Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Phire Tissue Direct PCR Master Mix

#F-170S **100 rxns**

Lot 00000000 **Expiry Date ____**

Danger.
Hazard statements:
May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements:
In case of inadequate ventilation wear respiratory protection. Avoid breathing dust/fume/gas/mist/vapours/spray. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Dispose of contents/container in accordance with local/regional/national/international regulations.
Thermo Fisher Scientific Baltics UAB, V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania, tel. +370 700 55131.

Store at -20°C

Extended version of product information is available online
www.thermoscientific.com/directpcr

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www.thermoscientific.com/onebio

COMPONENTS OF THE PRODUCT

Component	#F-170S 250 rxns x 20 µL 100 rxns x 50 µL	#F-170L 1250 rxns x 20 µL 500 rxns x 50 µL
2X Phire Tissue Direct PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL
Dilution Buffer	5 mL	2 × 12.5 mL
DNARelease Additive	3 × 100 µL	1.3 mL
Universal Control Primer Mix (25 µM each)	40 µL	40 µL
Water, nuclease- free	2 × 1.25 mL	10 × 1.25 mL
O'GeneRuler Express DNA Ladder	100 applications (50 µg)	

Shipping and storage

Upon arrival, store the components at −20 °C. The Dilution Buffer can also be stored at 4 °C once it is thawed.
Rev.5

1. Introduction

Thermo Scientific™ Phire™ Tissue Direct PCR Master Mix is designed to perform PCR directly from tissue samples with no prior DNA purification. Tissues such as mouse ear and tail, zebrafish fin, *Drosophila,* human hair are suitable starting materials. The samples can either be fresh or stored at −70 °C. A list of tissues tested with this Master Mix is available at www.thermoscientific.com/directpcr.
The Phire Tissue Direct PCR Master Mix contains reagents for two alternative protocols: Direct and Dilution & Storage protocols. The Master Mix is recommended for end point PCR protocols and it contains premixed gel loading dye which allows direct sample loading on the gel. The loading dye in the Master Mix does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation and restriction digestion.

2. Important Notes

- Detailed protocols for specific tissue samples are available on **www.thermoscientific.com/directpcr**
- Primer annealing temperatures with Phire are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 7.3 carefully.
- Add the sample directly into a PCR reaction instead of an empty tube.
- The Dilution & Storage protocol is recommended:
  - When working with new sample materials or a new primer pair.
  - With difficult samples or long amplicons.
  - When performing multiple reactions from the same sample.

3. PCR Protocol

Before starting read all Important notes (Section 2) and Sample handling guidelines (Section 4). The PCR setup can be performed at room temperature. **Always add the sample last to the reaction.** Read Section 4 carefully for sampling guidelines.

Table 1. Pipetting instructions (add items in this order)

Component	20 µL rxn	50 µL rxn*	Final conc.
H_2O	add to 20 µL	add to 50 µL	
2X Phire Tissue Direct PCR Master Mix	10 µL	25 µL	1X
Primer A	X µL	X µL	0.5 µM
Primer B	X µL	X µL	0.5 µM
Sample Direct Protocol: Dilution & Storage Protocol:	– 0.5 -1 µL	Amount depends on the sample** 1.25 - 2.5 µL	

* 50 µL reaction volume is recommended for the Direct protocol.
** 0.5 mm punch or a small sample of tissue (see www.thermoscientific.com/directpcr)

Table 2. Cycling protocol

Cycle step	2-step		3-step		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98 °C	5 min	98 °C	5 min	1
Denaturation	98 °C	5 s	98 °C	5 s	40
Annealing (see 6.3)	-	-	X°C	5 s	
Extension (see 6.4)	72 °C	20 s ≤1 kb 20 s/kb >1 kb	72 °C	20 s ≤1 kb 20 s/kb >1 kb	
Final Extension	72 °C +4 °C	1 min hold	72 °C +4 °C	1 min hold	1

Gel electrophoresis

2X Phire Tissue Direct PCR Master Mix contains a premixed gel loading dye. After PCR samples can be directly loaded on the electrophoresis gel for analysis.

Positive control reaction with purified DNA

When optimizing the direct PCR protocol, it is recommended to perform a positive control with purified DNA to ensure that the PCR conditions are optimal. If the positive control with purified DNA fails, the PCR conditions should be optimized until the control PCR gives a desired result. For troubelshooting refer to extended product information available online.

Negative control

It is recommended to use a no-template control with all Direct PCR assays to control contamination. To monitor the efficiency of cleaning the sampling tool, the cleaned tool can be dipped into the negative control sample. A second negative control performed without dipping the sampling tool is recommended to control for other sources of contamination.

4. Guidelines for Sample Handling

To obtain small and uniform samples, we recommend using 0.35 – 0.50 mm diameter puncher. If the puncher is to be reused, it is very important to clean the cutting edge properly to prevent cross-contamination between samples. Use 2% NaClO solution for cleaning and cross contamination prevention.
Other ways to take a sample is by cutting with scalpel to obtain 0.35 – 0.50 mm sample. Scalpel must be cleaned properly to prevent cross-contamination between samples.

5. Choosing the Protocol

This Master Mix is optimized for various tissue samples. **Please visit www.thermoscientific.com/directpcr to see a list of tested tissues.** With a few exceptions, both Direct and Dilution & Storage protocols are compatible with all sample types and applications. However, when amplifying longer fragments (e.g. > 500 bp from fish fin tissue or > 1 kb from other tissues) the Dilution & Storage protocol is recommended.

5.1 Direct Protocol

Direct Protocol: Take a sample of 0.5 mm in diameter from tissue by using the puncher or use a sterile scalpel to cut a very small piece of tissue (e.g. one *Drosophila* leg). Place the sample directly into the PCR reaction (50 µL of volume). It is recommended to place the sample into the liquid rather than into an empty tube. Make sure that you see the sample in the solution.

5.2 Dilution & Storage protocol

Before beginning, warm a heat block to 98 °C. Place the tissue sample into 20 µL of Dilution Buffer. Add 0.5 µL of DNARelease Additive. Mix by vortexing the tube briefly, and spin down the solution. If a larger sample is used, adjust the volume of the Dilution Buffer and DNARelease Additive accordingly. Make sure the sample is covered with the solution. Incubate the reaction for 2−5 minutes at room temperature and then place the tube into the pre-heated (98°C) block for 2 minutes. Spin down the remaining tissue and store the supernatant at −20 °C if not used immediately.
Usually 1 µL of supernatant is sufficient for a 20 µL PCR reaction. In some cases the supernatant may have to be diluted 1:10 or 1:100, or the PCR reaction performed in a 50 µL volume.

6. Notes About Reaction Components

6.1. Phire Tissue Direct PCR Master MIx

2X Phire Tissue Direct PCR Master Mix contains the dNTPs and provides 1.5 mM $MgCl_2$ concentration in the final reaction. The Master Mix employs Phire Hot Start II DNA Polymerase, that possesses the following activities:

5´→3´ DNA polymerase activity and a weak 3´→5´ exonuclease activity. When cloning DNA fragments amplified with Phire Hot Start II DNA Polymerase blunt end cloning is recommended.

6.2. Dilution & Storage Buffer

The Dilution & Storage Buffer has been optimized to release DNA from a wide variety of different tissues when supplemented with DNA Release Additive (see Section 6.3.). Samples in Dilution Buffer can be stored for up to 4 weeks in different temperatures (-20 °C, +4 °C and room temperature) before using in PCR. For long term storage, it is recommended to transfer the supernatant into a new tube and store at −20 °C.

6.3. DNARelease Additive

DNARelease Additive is required when PCR is performed directly from certain tissue samples using the Direct protocol. Cell debris present in these PCR products can cause DNA fragments to get trapped in the agarose gel wells. DNARelease Additive eliminates this problem. DNARelease Additive is also used in the Dilution & Storage protocol to improve the release of DNA from the tissue sample. Add 1.5 µL of DNARelease Additive into a 50 µL PCR reaction.

6.4. Primers

The recommendation for the final primer concentration is 0.5 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions on our website www.thermoscientific.com/tmc to determine the Tm values of primers and optimal annealing temperature.

7. Notes About Cycling Conditions

7.1. Initial denaturation

In Direct PCR protocols, the initial denaturation step is extended to 5 minutes to allow the lysis of cells, making genomic DNA available for PCR.

7.2. Denaturation

Keep the denaturation time as short as possible. Usually 5 seconds at 98 °C is enough for most templates. Note that the denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the thermal cycler.

7.3. Primer annealing

Note that the optimal annealing temperature for Phire Hot Start II DNA Polymerase may differ significantly from that of *Taq*-based polymerases. **Always use the Tm calculator and instructions on Thermo Scientific website www.thermoscientific.com/pcrwebtools to determine the Tm values of primers and optimal annealing temperature.** As a basic rule, for primers >20 nt, anneal for 5 seconds at a Tm +3 °C of the lower Tm primer. For primers ≤20 nt, use an annealing temperature equal to the Tm of the lower Tm prime. Two-step cycling without an annealing step is recommended for high-Tm primer pairs (Tm at least 69−72 °C).

7.4. Extension

The extension is performed at 72 °C. The recommended extension time is 20 seconds for amplicons ≤1 kb, and 20 s/kb for amplicons >1 kb.

8. Control Reactions

8.1. Direct PCR control reaction using the control primer mix

When using mammalian tissue samples (e.g. mouse, human tissue), we recommend performing Direct PCR control reactions with both Direct and Dilution & Storage protocols using the control primers supplied with this Master Mix. As a template, use the same tissue material as in the actual experiment. The universal control primer mix contains degenerate primers that amplify a 237 bp fragment of mammalian genomic DNA. The amplified region is a highly conserved non-coding region upstream of the SOX21 gene[1] and the primers are designed to amplify this region from a wide range of vertebrate species.

Each primer concentration is 25 µM.

Primer #1 (24-mer)

5'- AGCCCTTGGGGASTTGAATTGCTG -3'

Melting point: 73.5 °C

Primer #2 (27-mer)

5'- GCACTCCAGAGGACAGCRGTGTCAATA -3'

Melting point: 72.2 °C (R=A), 75.3 °C (R=G)

Please note that these control primers are not compatible with fish or insect samples. The recommended control primer sequences for *Drosophila* and zebrafish are available at www.thermoscientific.com/directpcr.

Table 3. Pipetting instructions for control reactions.

Component	20 µL rxn	50 µL rxn*
H_2O	add to 20 µL	add to 50 µL
2X Phire Tissue Direct PCR Master Mix	10 µL	25 µL
Universal control primer mix	0.4 µL	1 µL
Samples Direct Protocol: Dilution & Storage Protocol:	– 1 µL	Amount depends on the sample** 2.5 µL

*50 µL volume is recommended for Direct protocol.

**0.5 mm punch or a small sample of tissue (see www.thermoscientific.com/directpcr)

Table 4. Cycling instructions for control reactions using primers included.

Cycle step	Temp.	Time	Cycles
Initial denaturation	98 °C	5 min	1
Denaturation Annealing/Extension	98 °C 72 °C	5 s 20 s	40
Final Extension	72 °C 4 °C	1 min hold	1

CERTIFICATE OF ANALYSIS

Performance in PCR is tested by the amplification 7.5 kb fragment from human genomic DNA.

Absorption measured at 424 nm and 614 nm.

Quality authorized by: Jurgita Zilinskiene

REFERENCES

1. Woolfe A. *et al.*(2005) *PLoS Biology3*: 116–130.

Troubleshooting

To optimize Direct PCR three key steps have to be considered: dilution protocol, sample size and optimal primer annealing temperature.

Troubleshooting information is available in the extended version of the protocol. See www.thermoscientific.com/directpcr for more details.

SAFETY INFORMATION

DNARelease Additive

Danger

Hazard statements:

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements:

P285 In case of inadequate ventilation wear respiratory protection.

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.

P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

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